Mutation Frequency in Mouse Embryonic Stem Cells After Exposure to Carbon Nanomaterials

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Introduction

Carbon nanomaterials consist of a broad variety of synthetic materials of carbon with at least one dimension less than100nm in length.

Unique properties emerge at the nanoscale level:

- Increased surface area & mechanical strength
- Altered physicochemical reactivity, solubility & surface charge
- Enhanced electrical and thermal conductivity

These properties have led to valuable applications within industrial, electronic, optic, biomedical, and environmental domains. Thus, human exposure to carbon nanomaterials is increasing. Currently, there exists a great need to investigate potential health hazards associated with these materials.

Previous studies indicate cytotoxic and genotoxic effects of these materials to mammalian cells.

Materials

- Carbon Nanofibers (CNF): T1 and T3
- Carbon Nanotubes (CNT): CNT1 and CNT2
- Multi-walled Carbon Nanotubes (MCNT)

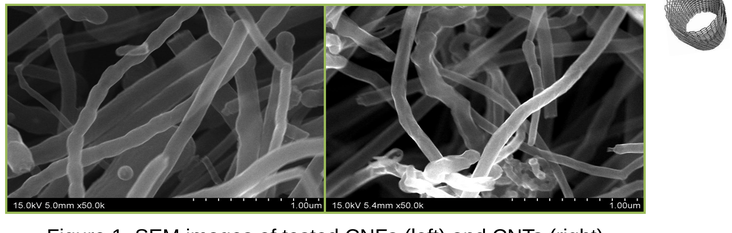


Figure 1. SEM images of tested CNFs (left) and CNTs (right).

Hypothesis

Carbon nanomaterial exposure will result in increased mutation frequency in MES cells compared to spontaneous mutation frequency.

Methods

Aprt +/- 3C4 MES cells cultured. Adenine phosphoribosyltransferase (Aprt), purine salvage enzyme used as a reporter for mutation frequency, which is determined by Aprt-deficient colonies.

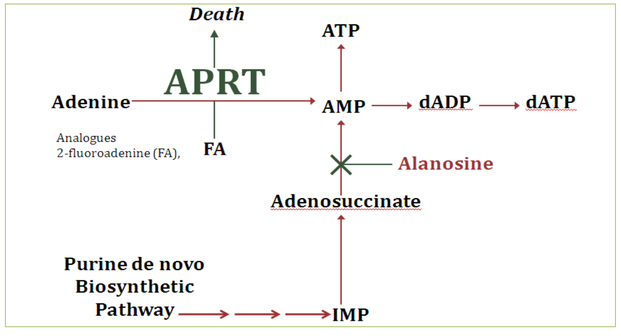


Figure 2. Biochemistry of the APRT system.

- Adenine/Alanosine treat: eliminate pre-existing Aprt-deficient cells.
- Nanomaterial treatment: 5ug/ml; 72h exposure.
- Cell count: seed 5 10^5 cells/MEF plate; replicates of 10.
- 2-FA treatment: Select for loss of Aprt function
- Colony formation: 15 days before staining.

$$\text{Mutation Frequency} = \frac{\text{Number colonies formed after selection}}{\text{Number of cells initially seeded}} \times \text{CFE}$$

Results

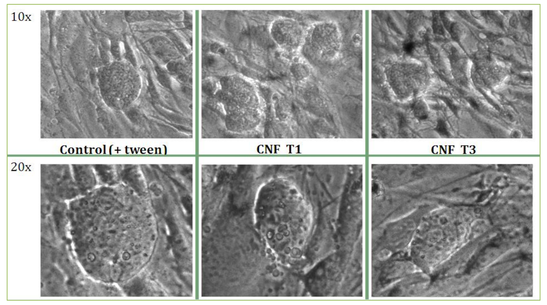
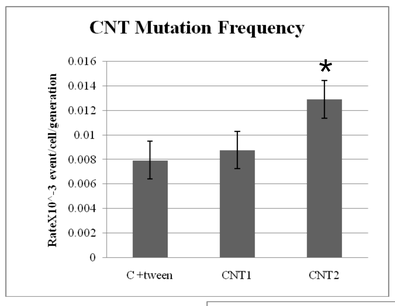
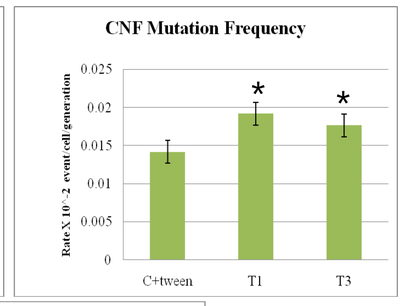
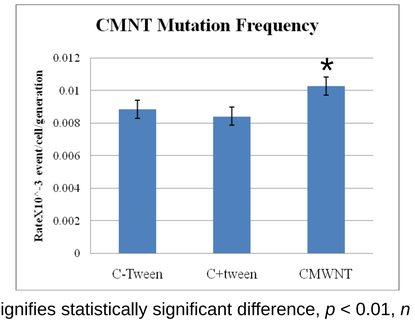


Figure 3. Phase contrast: changes in MES cell morphology post-treatment







*Signifies statistically significant difference, $p < 0.01$, $n = 10$.

Mutation frequency increase: CNT2 = 62%
CNF T1 = 36%; CNF T3 = 25%; CMNT = 16%

Discussion

- Demonstration of mutagenic properties
- Future directions: characterize mutational events, chronic exposure study, elucidate biological response mechanism